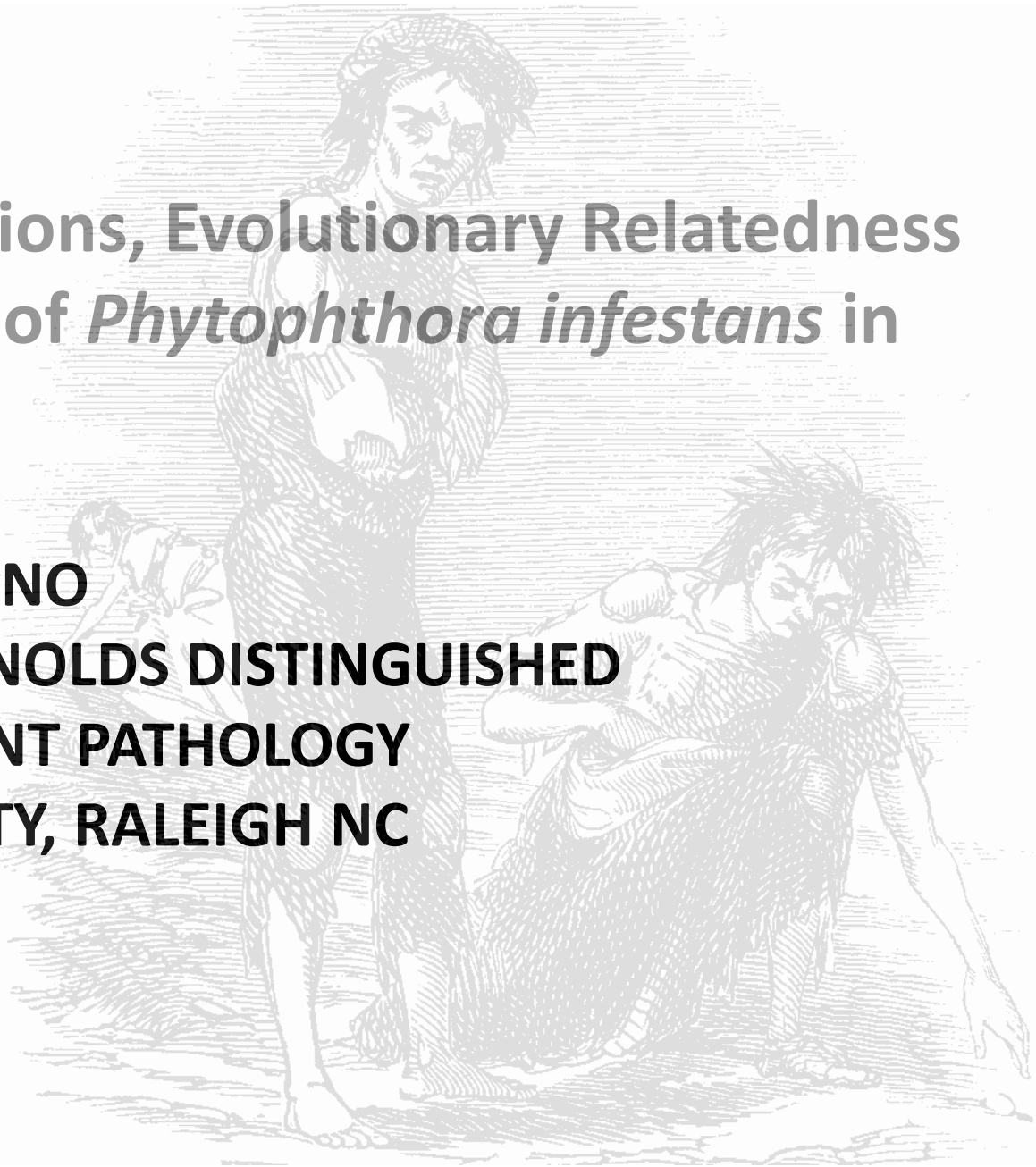
GLOBAL FOOD SECURITY

NC STATE UNIVERSITY

Worldwide Migrations, Evolutionary Relatedness and Reemergence of *Phytophthora infestans* in Asia



JEAN BEAGLE RISTAINO
WILLIAM NEAL REYNOLDS DISTINGUISHED PROFESSOR OF PLANT PATHOLOGY
NC STATE UNIVERSITY, RALEIGH NC

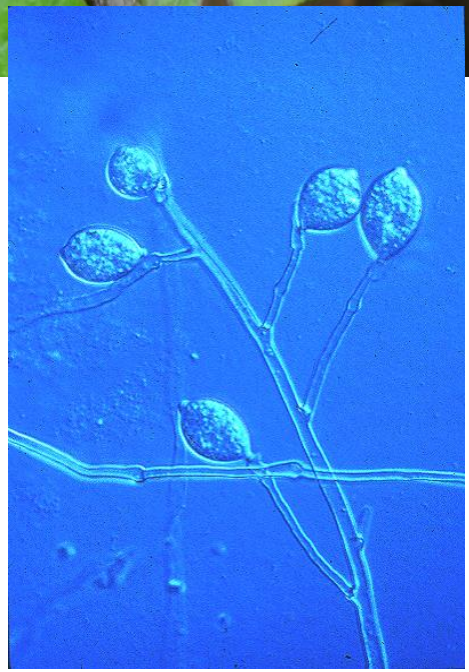
NC STATE UNIVERSITY

A constraint to potato production and global food security worldwide

Has increased in incidence, geographical and host range

Spread of *Phytophthora infestans*

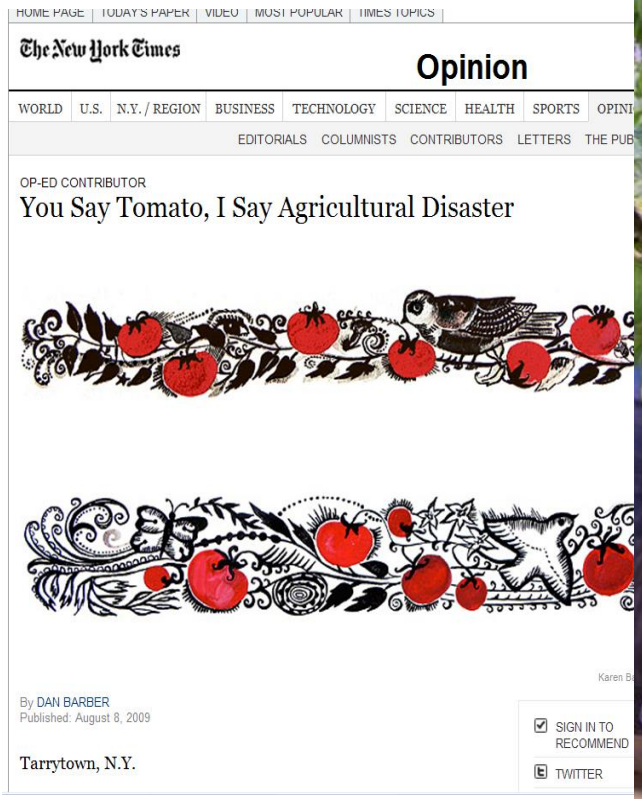
Spread by sporangia – spread in air – 100’ kilometers



Phytophthora infestans sporangia

Late blight epidemics in the US in 2009
Emergence of US-22 strain

- Climate change – rainy season
- Movement of infected tomato transplants
- Susceptible varieties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The New York Times

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OP-ED CONTRIBUTOR

You Say Tomato, I Say Agricultural Disaster

By DAN BARBER
Published: August 8, 2009

Tarrytown, N.Y.

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Science News

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Late Blight -- Irish Potato Famine Fungus -- Attacks U.S. Northeast Gardens And Farms Hard

ScienceDaily (July 5, 2009) — Home gardeners beware: This year, late blight -- a destructive infectious disease that caused the Irish potato famine in the 1840s -- is killing tomato and potato s in gardens and on commercial farms in the ern United States. In addition, basil downy w is affecting plants in the Northeast.

"Late blight has never occurred this early and this widespread in the U.S," said Meg McGrath, associate professor of plant pathology and plant-microbe biology.

One of the most visible early symptoms of the disease is brown spots (lesions) on stems. They begin small and firm, then quickly enlarge, with white fungal growth developing under moist conditions that leads to a soft rot collapsing the stem.

Classic symptoms are large (at least nickel-sized) olive-green to brown spots on leaves with slightly fuzzy white fungal growth on the underside when conditions have been humid (early morning or after rain). Sometimes the of the spot is yellow or has a water-soaked appearance. begin tiny, irregularly shaped and brown. Firm, brown spots p on tomato fruit.

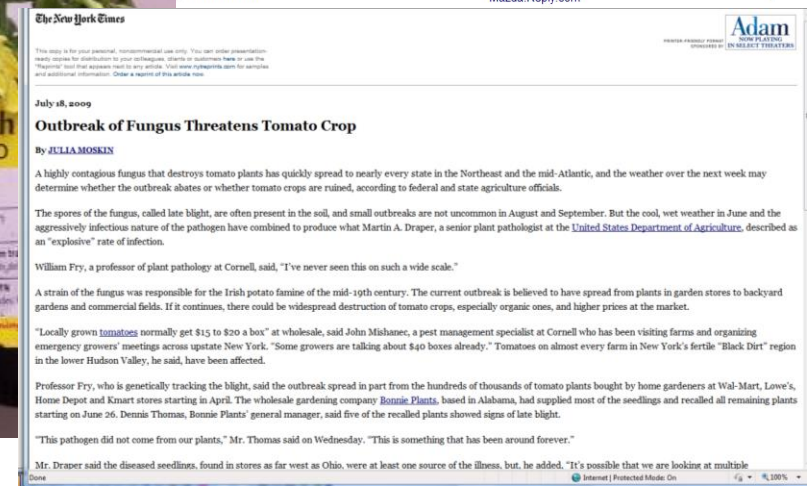
enlarge

Leaf lesions due to late blight. (Credit: Copyright College of Agriculture and Life Sciences, Cornell University)

Ads by Google

Plant Problems? Try BASF
Control downy mildew diseases with BASF Stature SC fungicide
www.BetterPlants.BASF.us

2009 Mazda Clearance
Inventory blowout pricing!
Dealers are liquidating inventory
Mazda.Reply.com


The New York Times

Adam

July 18, 2009

Outbreak of Fungus Threatens Tomato Crop

By JULIA MOSKIN

A highly contagious fungus that destroys tomato plants has quickly spread to nearly every state in the Northeast and the mid-Atlantic, and the weather over the next week may determine whether the outbreak abates or whether tomato crops are ruined, according to federal and state agriculture officials.

The spores of the fungus, called late blight, are often present in the soil, and small outbreaks are not uncommon in August and September. But the cool, wet weather in June and the aggressively infectious nature of the pathogen have combined to produce what Martin A. Draper, a senior plant pathologist at the United States Department of Agriculture, described as an "explosive" rate of infection.

William Fry, a professor of plant pathology at Cornell, said, "I've never seen this on such a wide scale."

A strain of the fungus was responsible for the Irish potato famine of the mid-19th century. The current outbreak is believed to have spread from plants in garden stores to backyard gardens and commercial fields. If it continues, there could be widespread destruction of tomato crops, especially organic ones, and higher prices at the market.

"Locally grown tomatoes normally get $15 to $20 a box" at wholesale, said John Mishanec, a pest management specialist at Cornell who has been visiting farms and organizing emergency growers' meetings across upstate New York. "Some growers are talking about $40 boxes already." Tomatoes on almost every farm in New York's fertile "Black Dirt" region in the lower Hudson Valley, he said, have been affected.

Professor Fry, who is genetically tracking the blight, said the outbreak spread in part from the hundreds of thousands of tomato plants bought by home gardeners at Wal-Mart, Lowe's, Home Depot and Kmart stores starting in April. The wholesale gardening company Bonnie Plants, based in Alabama, had supplied most of the seedlings and recalled all remaining plants starting on June 26. Dennis Thomas, Bonnie Plants' general manager, said five of the recalled plants showed signs of late blight.

"This pathogen did not come from our plants," Mr. Thomas said on Wednesday. "This is something that has been around forever."

Mr. Draper said the diseased seedlings, found in stores as far west as Ohio, were at least one source of the illness, but, he added, "It's possible that we are looking at multiple

Objective: improve management tools

Public reports

National reporting & alert website

Co-PD labs

P. infestans genotypes (SSRs) & phenotypes





enhanced Decision Support System

<u>Management recommendations</u> fungicide use, including solutions for organic growers

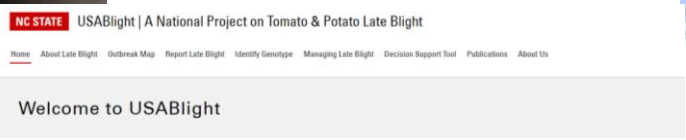
10% reduction in fungicide use = $20 million/year

Slide courtesy of H Judelson

USABlight.org –Disease alerts

Sample Submission

Alerts and Mapping



New Diagnostics

Decision Support Tool

DSS Daily Email Report

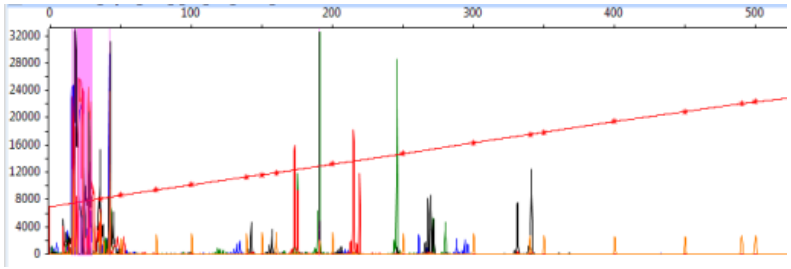
Mills River / ASHEVILLE

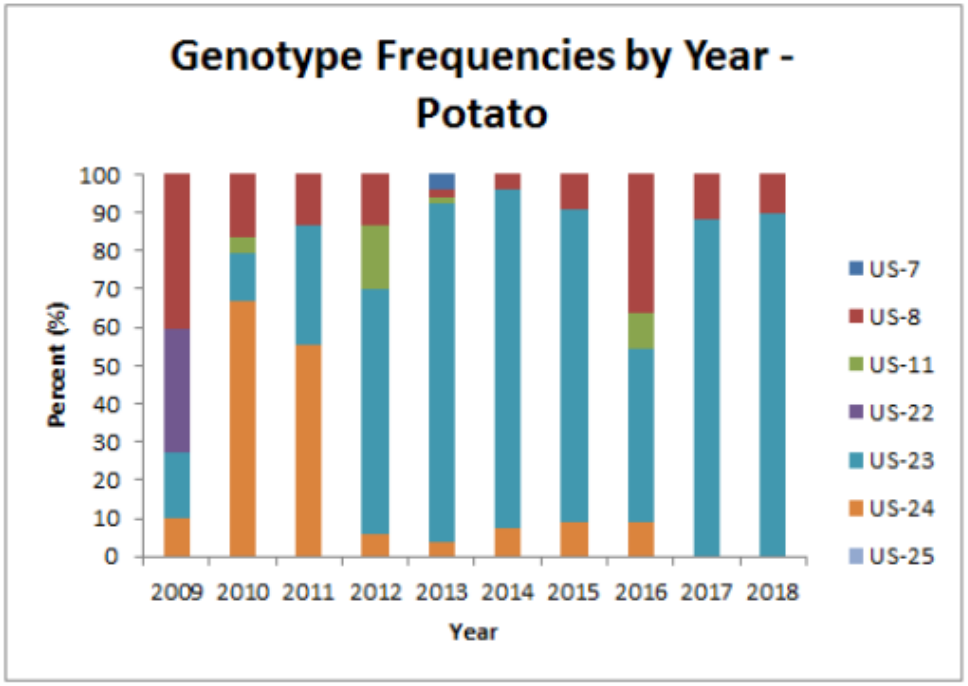
Date[1]	8/16	8/17	8/18	8/19	8/20	8/21	8/22	8/23
Blight Units	303	309	316	323	330	337	344	351
Fungicide Units	-91	-92	-94	-96	-97	-98	-99	-100

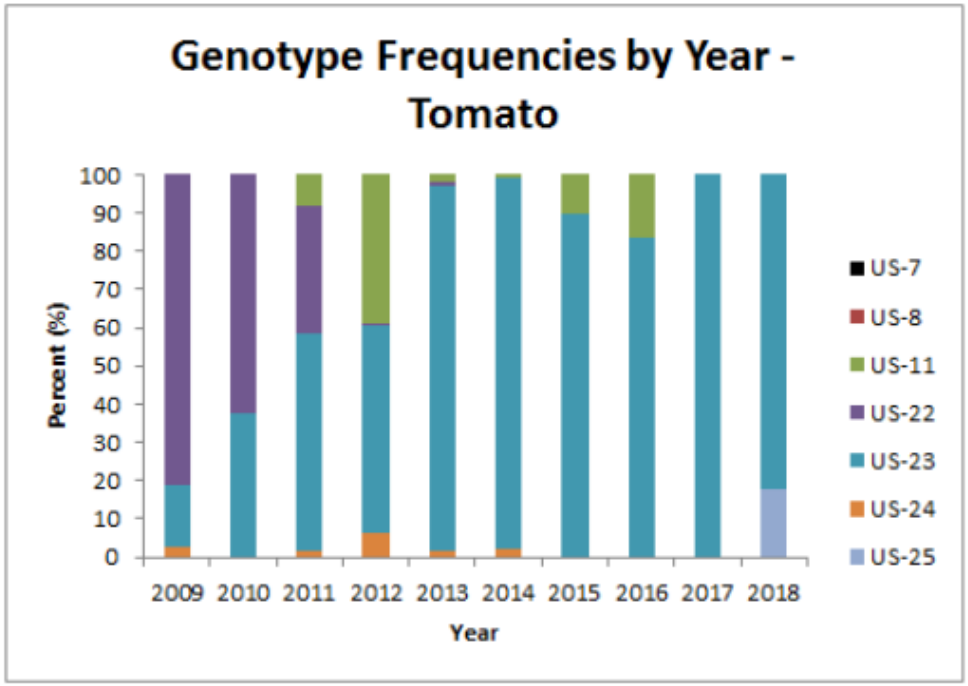
Key:

below threshold	
blight unit threshold exceeded	>=30
fungicide unit threshold exceeded	<=-15

Genotyping

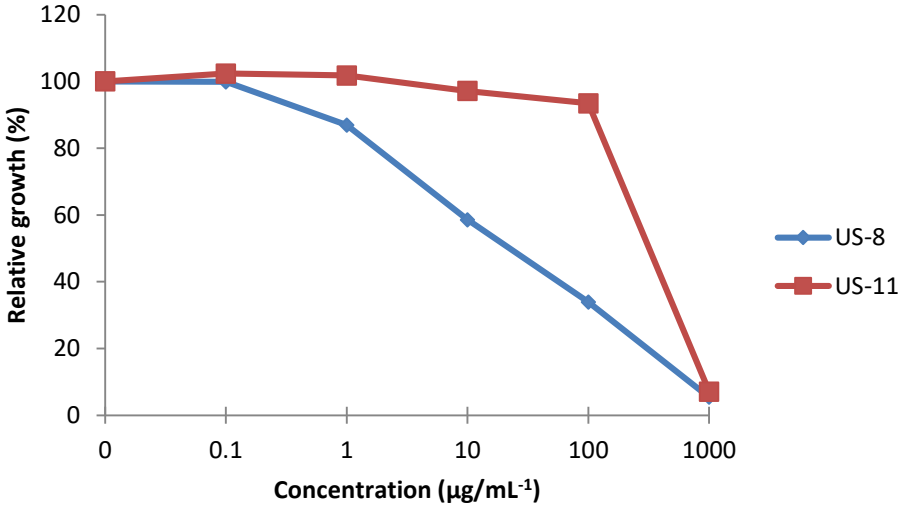


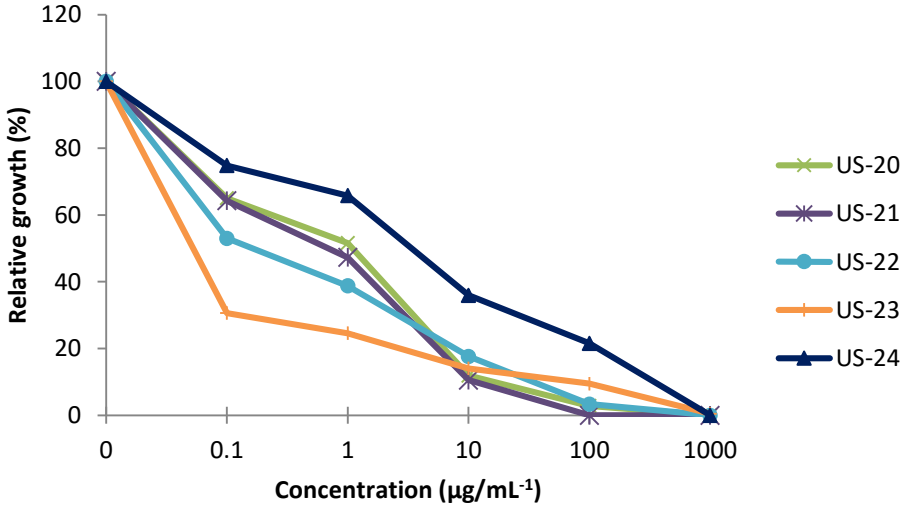




- US-22 caused 2009 outbreaks
- displaced completely by US-23 on both hosts
- US-8 declined on potato

Mefenoxam Sensitivity by lineage



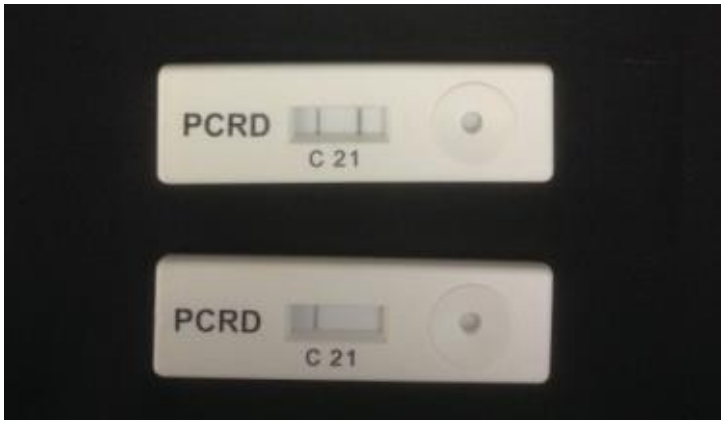


-US-8 and US-11 resistant to mefenoxam

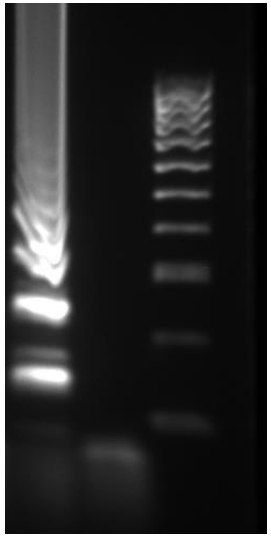
All lineages sensitive to: azoxystrobin, cyazofamid, cymoxanil, fluopicolide, mandipropamid

P. infestans LAMP Assay

- Ideal for diagnostic protocols
  - LAMP primers designed specific for P infestans
  - Rapid protocol can be adapted to field situations
  - Amplification product can be visualized in the field with visual nucleic acid stains (e.g. SYBR green or HNB)
  - Can be adapted to lateral flow devices(LFDs)



Samples with SYBR green. The three samples on the left are positive

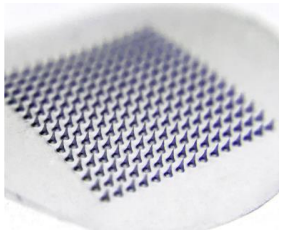


Example of positive and negative LAMP reaction. Ladder is 100bp.

Sensors for plant disease detection in the field

Jean Ristaino Qingshan Wei

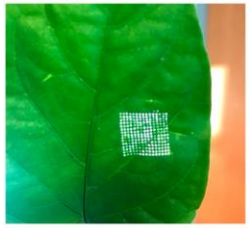
NC STATE UNIVERSITY

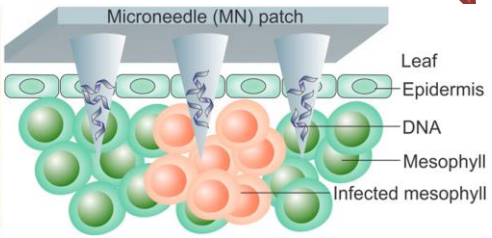


Volatile Organic Compounds (VOCs)

Pathogen DNA extraction

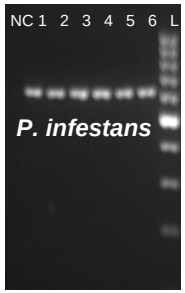
Noninvasive Plant Volatile Profiling



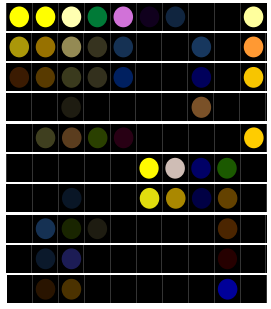


Mobile phone reader

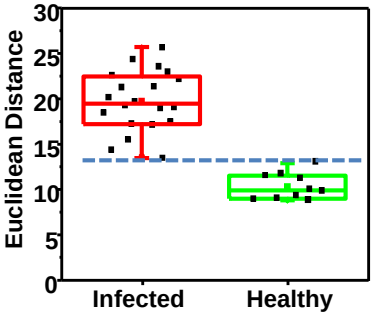
Chemical sensor array



Classification



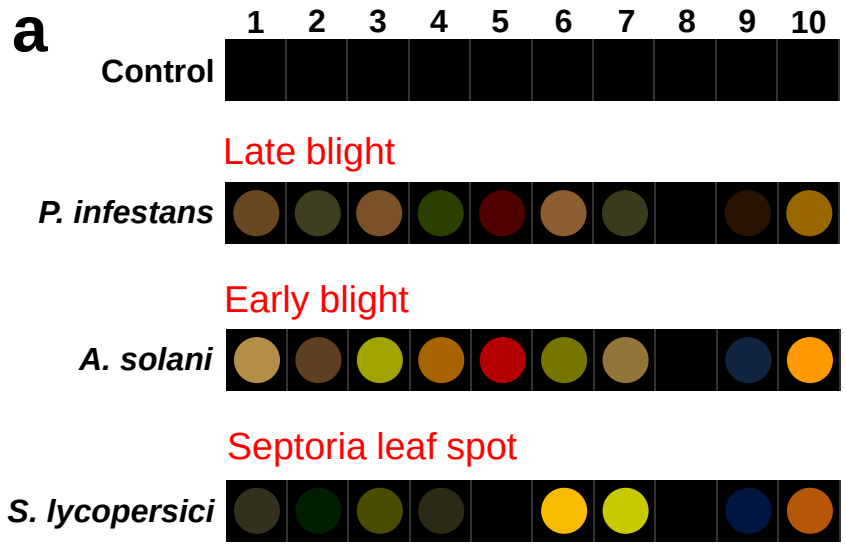
Diagnosis

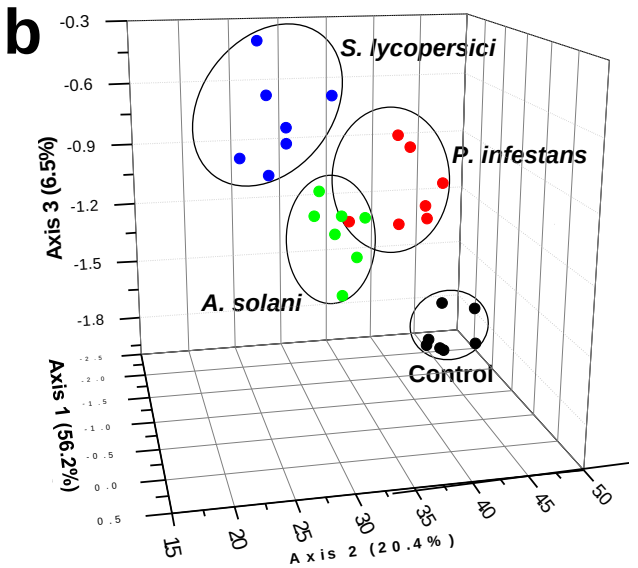


Paul, R. et al. 2019. ACS Nano 13:6540-6549

Li et al, 2019. Nature Plants 5:856-866 .

We can differentiate three tomato pathogens by VOC's before symptoms



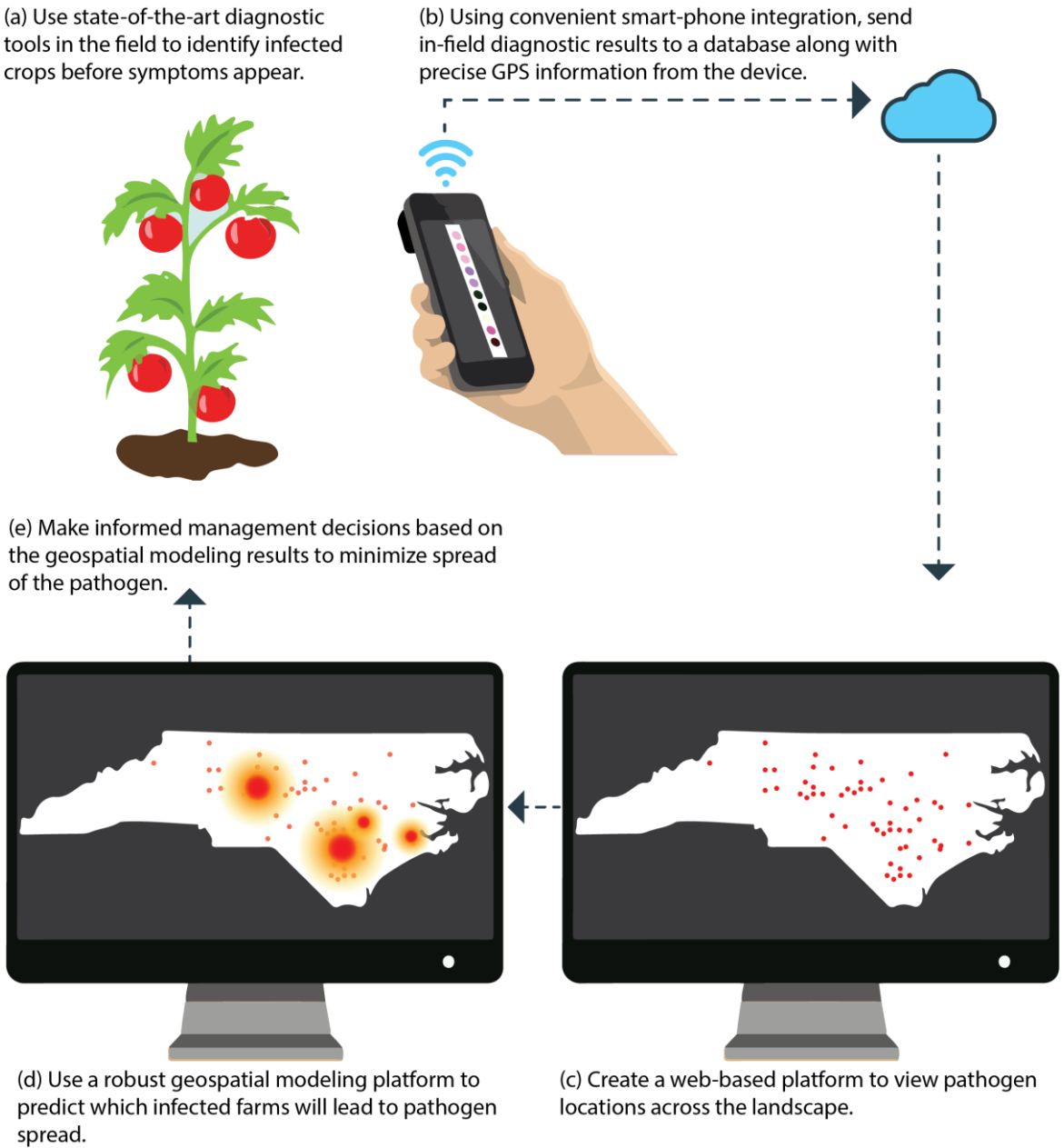


Classification accuracy: ~93%

(2 errors out of 28 samples tested in total)

Li, Z. et al., 2019. Noninvasive Plant Disease Diagnostics Enabled by Smartphone-Based Fingerprinting of Leaf Volatiles. Nature Plants 5:856-866

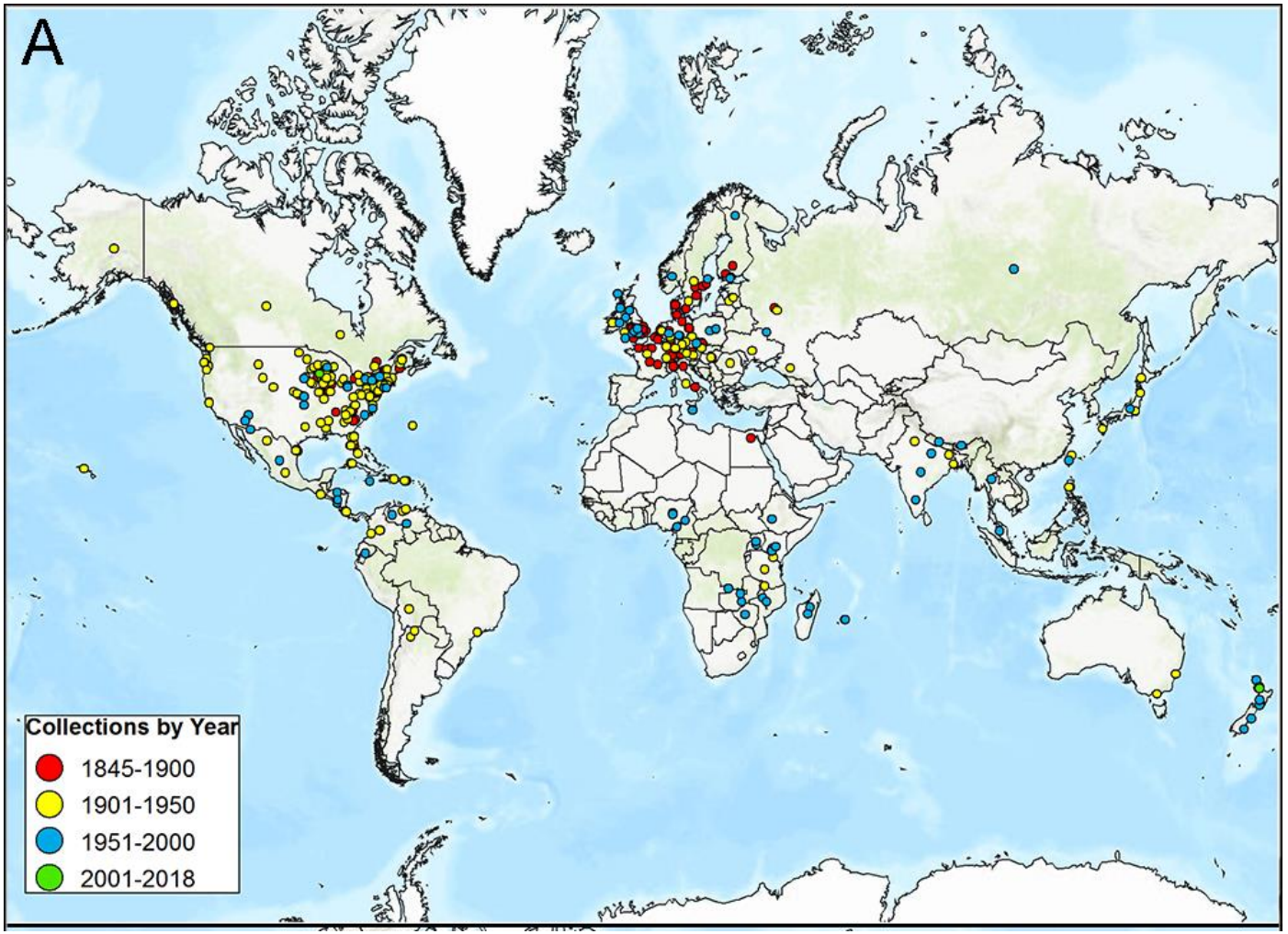
Sensor based late blight mapping



Big questions about historical *P. infestans*

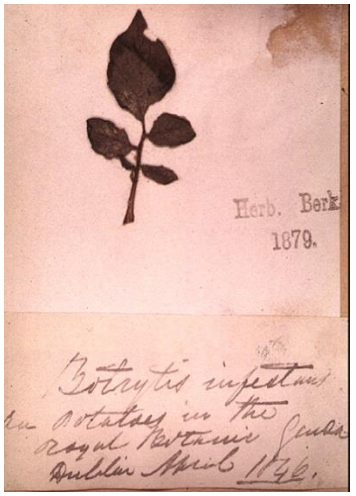
- What lineages are circulating in US today and where are they from?
- What lineage caused the famine?
- Where did the famine lineage come from?
- Has this pathogen always had a large genome?
- Has effector diversity changed with time?
- Are historical genotypes still circulating?
- Did same lineage cause disease in the US and Europe?
- How far did the famine lineage migrate?

Over 1200 samples of *P. infestans* in mycological herbaria



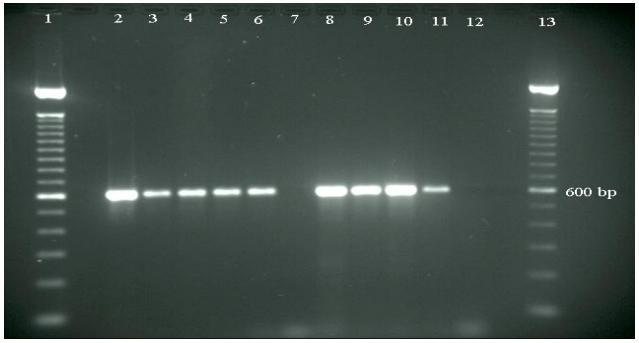
Did US-1 lineage cause the famine?

- Herbarium specimens: use for the first time to study historical late blight epidemics.
- Historic late blight was not caused by Ib lineage – but a Ia lineage – The Ib lineage emerged later
- Multiple mt lineages in Central America in mid 20th century



Ristaino et al, 2001, Nature 41:695-697

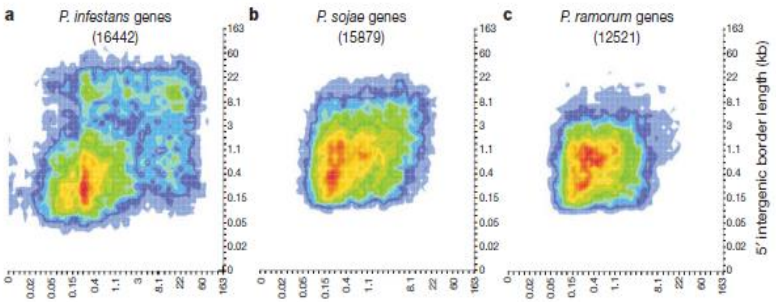
May and Ristaino, 2004, Mycol. Res. 108:171-179.



Isolate	Haplo	Date	*MspI restriction site*
93-3	1a	1993	AATTTCTCCAACAAAACTACTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
188.1.1	1b	1996	AATTTCTCCAACAAAACTACTTGAACCCGGAATAGACATATTTGCTAATACATAAATAAA
18-94	11a	1996	AATTTCTCCAACAAAACTACTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
94-52	11b	1994	AATTTCTCCAACAAAACTACTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
Herbarium Spec.Number		Date	
6		1847	AATTTCTCCAACAAAA-T-CTT-AACCTGGAATAGACATATTTGCTAATACATAAATAAA
22		1875	AATTTCTCCAACAAAACTACTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
33		1886	AATTTCTCCAACAAAACTACTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
31		1884	NATTTCTCCACC-NAANTT-TTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
186882		1902	AATTTCTCC-ACA---CC-CTTGAAC---G-ATTGACATAT-TGCTAAT-CAT--ATAAA
186890		1906	AATTTCT-CNAC-AA-CC-CTTGANCCTGGAATAGACATATTTGCTNATACATAAATAAA
186875		1916	AATTTCTCCAACA-AACTAC-TGNACNTGGNAT-GACATATTTGCTAATACATAAATAAA
186864		1919	AATTTCT-C-AC-AAACTACTT-AACCTNGAAT-GACATATT--CTA--NCAT-N-T-AA
186869		1922	AATTTCTCCN-CAAAA-A-CTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
186872		1928	AATTTCTCCAACAAAACTACTTGAACCTGGAATAGACATATTTGCTAATACATAAATAAA
			****** * * * * ** ******* ** *** * **

Pathogen Genome Sequenced

- Genome is highly expanded –repetitive - 75%
- **Effector** diversity – Avirulence proteins needed to overcome host resistance
- What is driving expansion? Breeding?
- Pathogen/population genomics and database



Haas, B. J., et al. 2009. Nature 461:393-398

How different is modern *P. infestans* genome from the genomes of historic *P. infestans*

N.° 1198. Botrytis fallax DESMAZ.

DESMAZ. *Pl. crypt. de France*, n.° 1492. — Kx. *Rech. Fl. crypt. des Fland.*, 3e *cent.*, *pag.* 45, *n.*° 85. — Botrytis infestans *Mont.* — Botrytis vastatrix *Lib.* — Pritchardia solani *Muhlenb.* — Choléra de la Pomme de terre.

Sur les feuilles languissantes du *Solanum tuberosum*, aux environs d'Audenarde. (M.r TOSQUINET.)

Genome evolution of *P. infestans* Collaboration with Univ. Copenhagen



Mike Martin
and Tom Gilbert

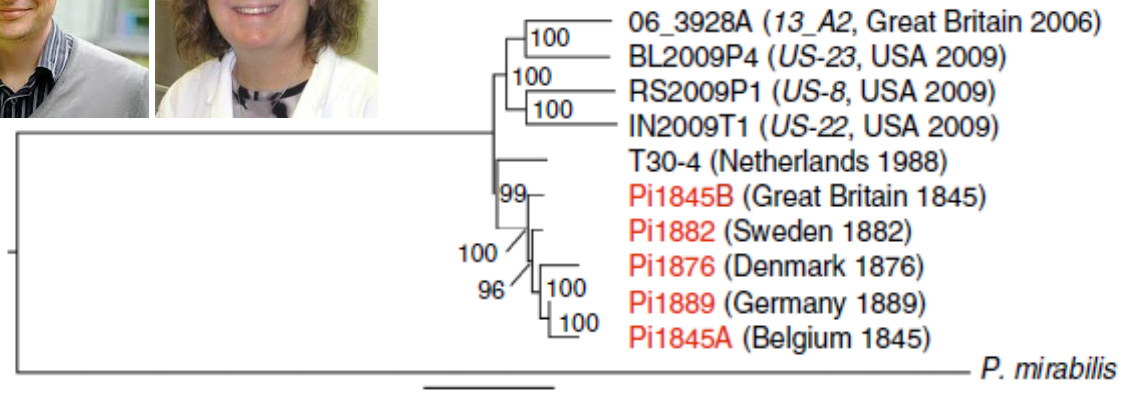


Figure 1 | Maximum-likelihood phylogram of *P. infestans* genomes from the first historic outbreaks of disease and later outbreaks. Nodes are labelled with their support values from 100 bootstrap replicates. The scale bar indicates a branch length of 0.2 nucleotide substitutions per site.

- Highly supported monophyletic clade for historic samples
- Sequenced US-8, US-22 and US-23 lineages
- T30-4 intermediary position and modern genomes in a distinct clade
- Samples from modern and historic time periods differed by > 120,000 SNP's
- Pi1845 and Pi 1889 differed by > 12,000 SNP's
- Differences between the European historical samples from the 1840s and the 1870s and 1880s **suggest that the pathogen was brought to Europe more than once.**

Martin, M. D. et al. 2013. Nat. Commun. 4:2172 doi: 10.1038/

Fewer effectors in historic samples

- Effectors deleted in gene sparse regions
- Avirulence genes considered important for pathogenesis were absent in historic genomes.
- **Expansion** of effectors over time
- Virulent form of *Avr2* and *Avr3a* were absent in historic samples
- In the areas of the genome that today control virulence, there was little similarity with historical strains, suggesting that the pathogen has evolved in response to human actions like breeding disease-resistant potatoes.

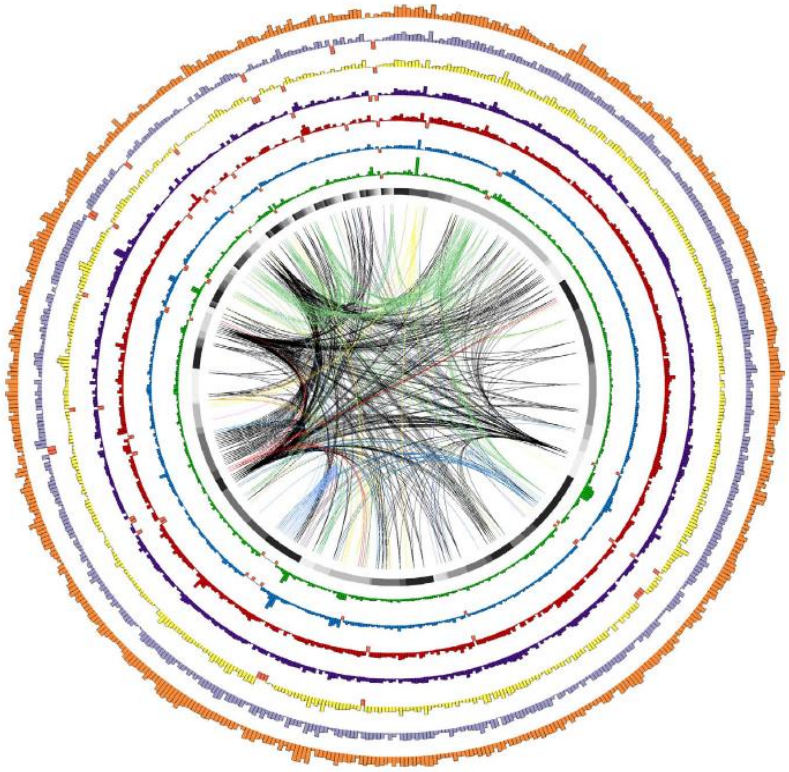
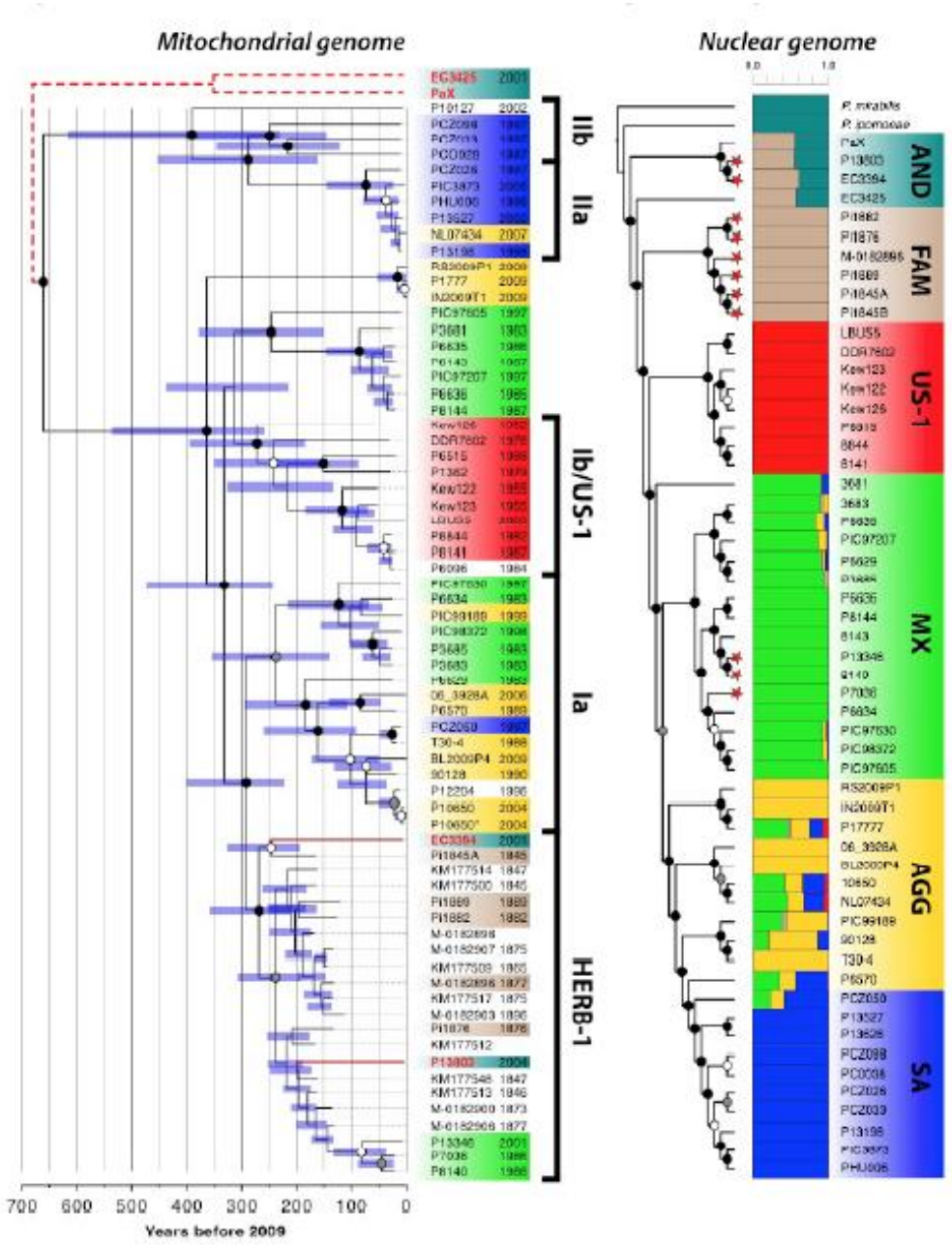


Figure 2 | Visualization of sequencing coverage distribution across all reference RXLR effectors. Bar heights represent the mean-normalized coverage of 583 reference RXLR effector genes in the resequenced genome of a particular sample).



Genomic characterization of South American *Phytophthora* hybrid mandates reassessment of geographic origin of *Phytophtora infestans*.
Martin et al, 2015. Mol. Biol. Evol. 33:478-491

Mitogenomes

- **Herb-1 lineage persists in *P andina*** (Ia) from *S. betaceum* (red)
- Two distinct Herb-1 mt lineages –diverged
- Herb-1 mtDNA lineage not strictly associated with FAM lineages of *P. infestans*

Nuclear genomes - 6 lineages

- *P. andina* shows mixed ancestry with famine lineages and outgroup species indicating hybrid, basal in tree
- Famine era lineages form highly supported sister clade at base of tree
- US-1 and Mexican lineages diverged later
- Modern Mexican lineages and US aggressive lineages – admixture- MX likely source of some AGG lineages
- Modern SA lineages (EC-1, PE-3, BR-1) from domesticated potato most derived
- Probably ancestral lineages of the pathogen exist on wild *Solanum* hosts in SA

Did 19th century *P. infestans* in the US migrate to Europe?

- Late blight first to US in 1843
- Reports in Europe and Ireland by the fall 1845

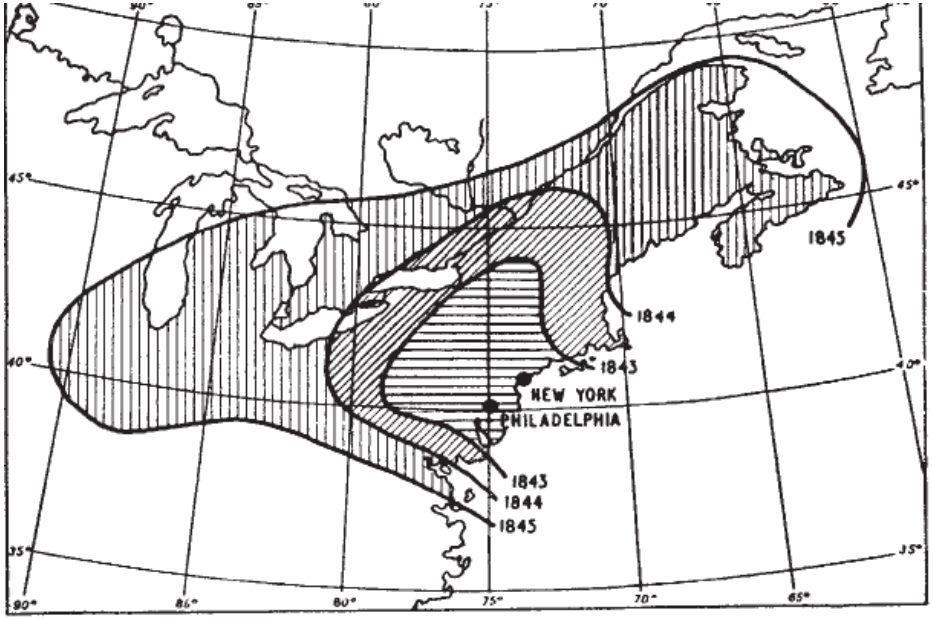


Fig. 1. Approximate extent of potato blight attacks in the United States and Canada during 1843–45 (after Stevens)

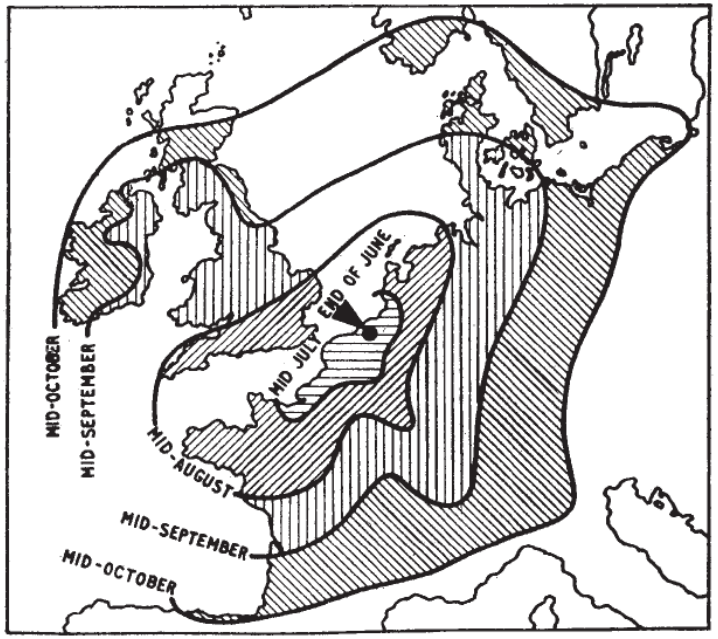
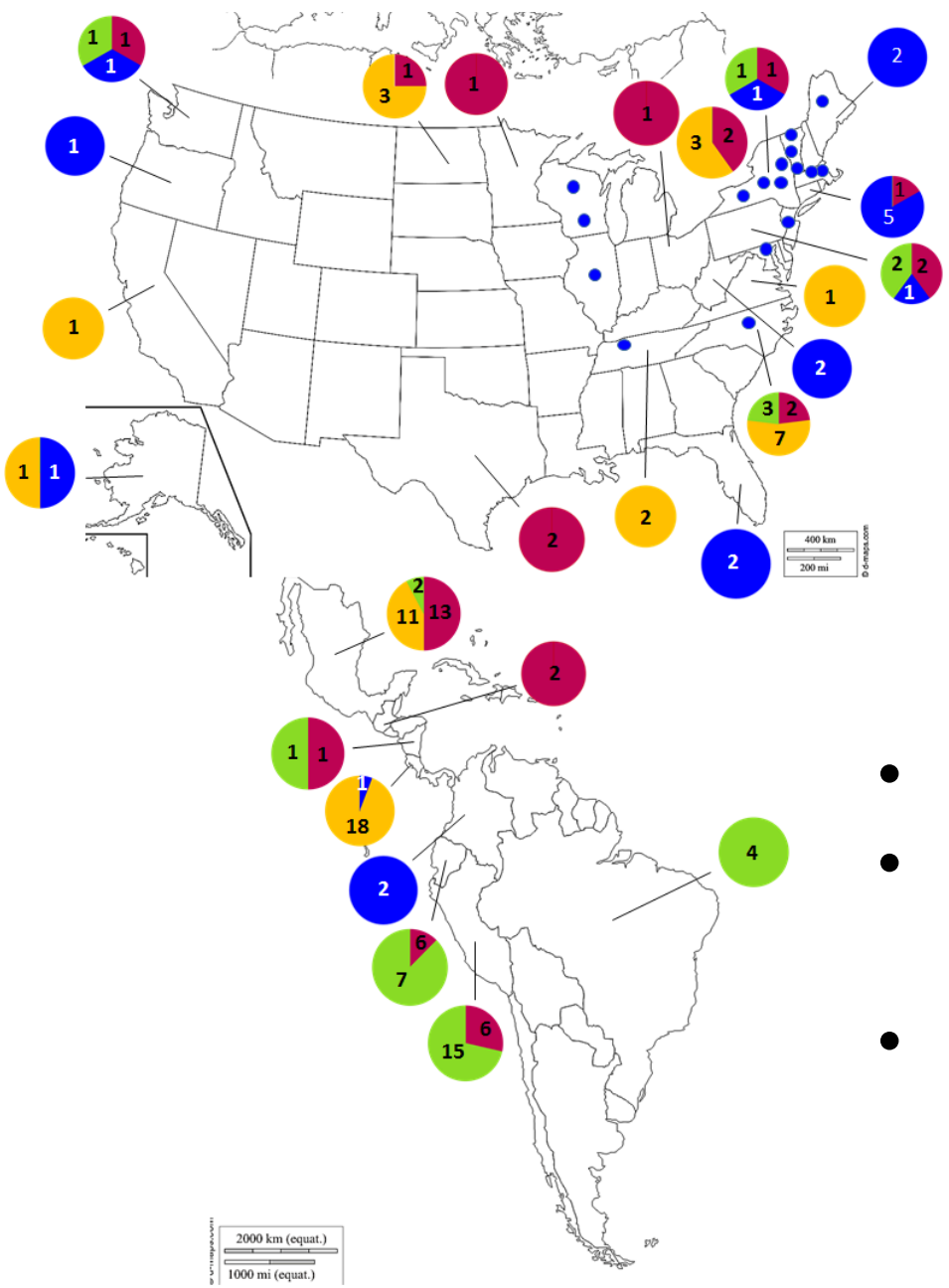


Fig. 2. Approximate dates of first report of potato blight in Europe in 1845

12 Plex SSR data -modern and historic US/SA samples

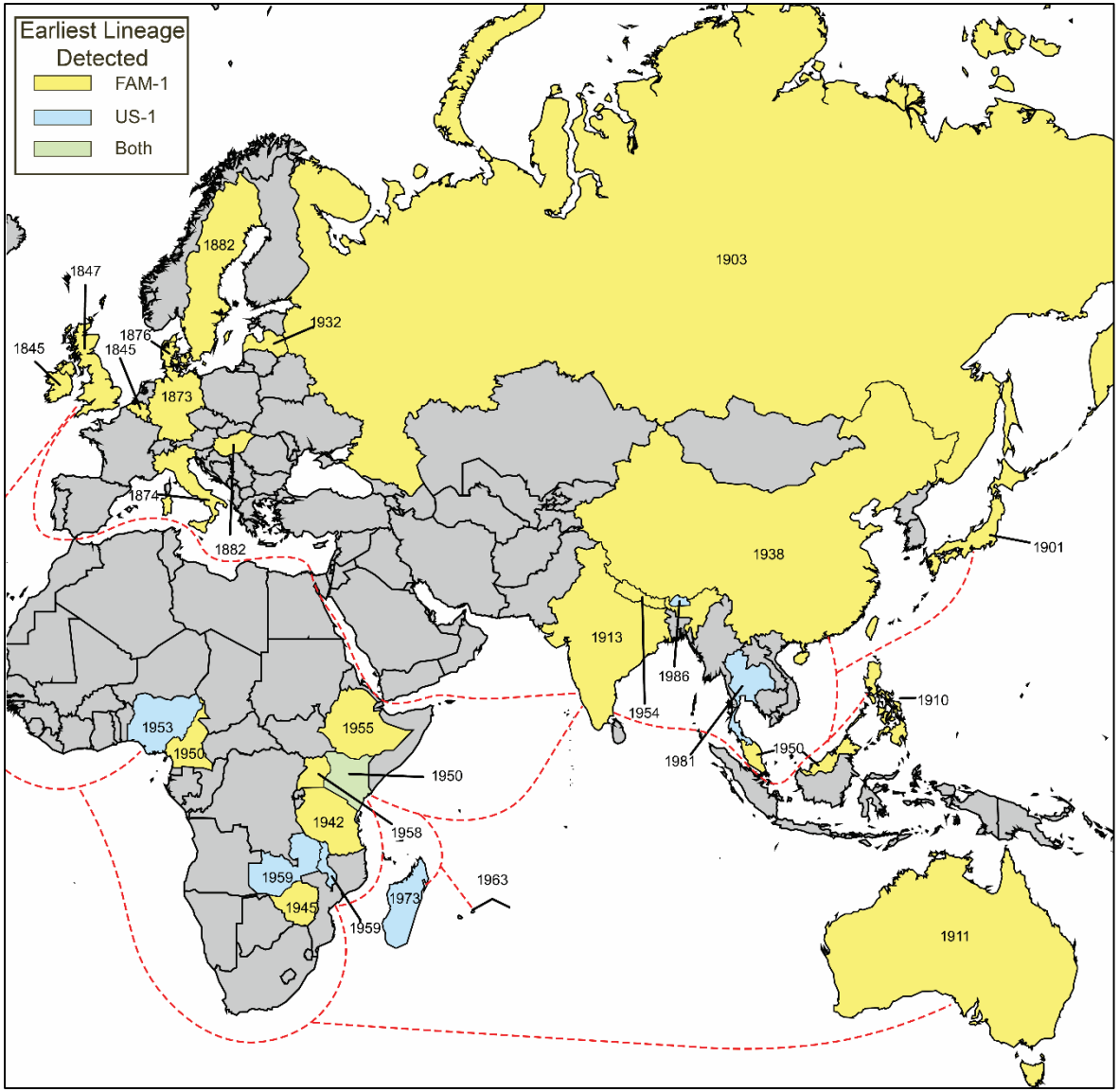
- **US/EU historic lineages** cluster into one group –named FAM-1 lineage
- The oldest South American samples from **Colombia** share allelic similarity with historic US and EU populations.
- **US-1 (Ib) lineages** forms a second group
- **Modern South American (SA) and Irish lineages** form a third group.
- The US-23 lineage clusters with SA lineages
- **Central American, Mexican and Modern US aggressive lineages** (US-6, 7, 8, 11, 22, 24) share allelic diversity with Mexican lineages suggesting a Mexican origin of these recent lineages into the US

Plos One December 28, 2016



- FAM-1 lineage – not extinct.
- Remained in US for 100 years.
- Same lineage caused disease in US and Europe during famine and into early 20th century

Did FAM-1 lineage migrate from Europe to other continents? YES!



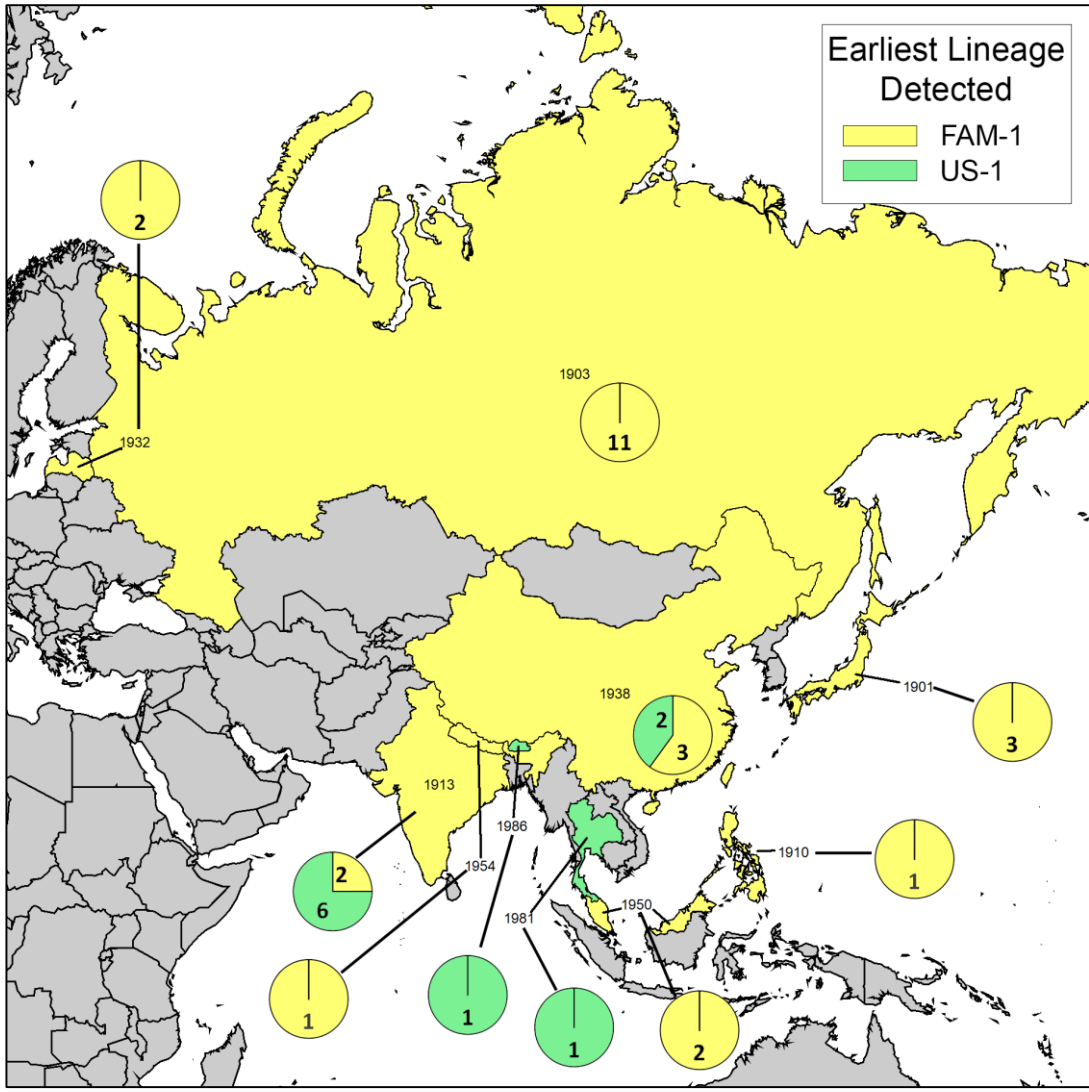
First lateblight outbreaks in Asia

Earliest known confirmed occurrences of FAM-1

- Japan- 1901
- Russia – 1903
- The Philippines – 1910
- India – 1913
- Latvia- 1932
- China- 1938
- Malaysia- 1950
- Nepal- 1954

US-1- 1950s

- China- 1950
- India -1968
- Thailand – 1981
- Bhutan – 1986
- Malaysia - 1987

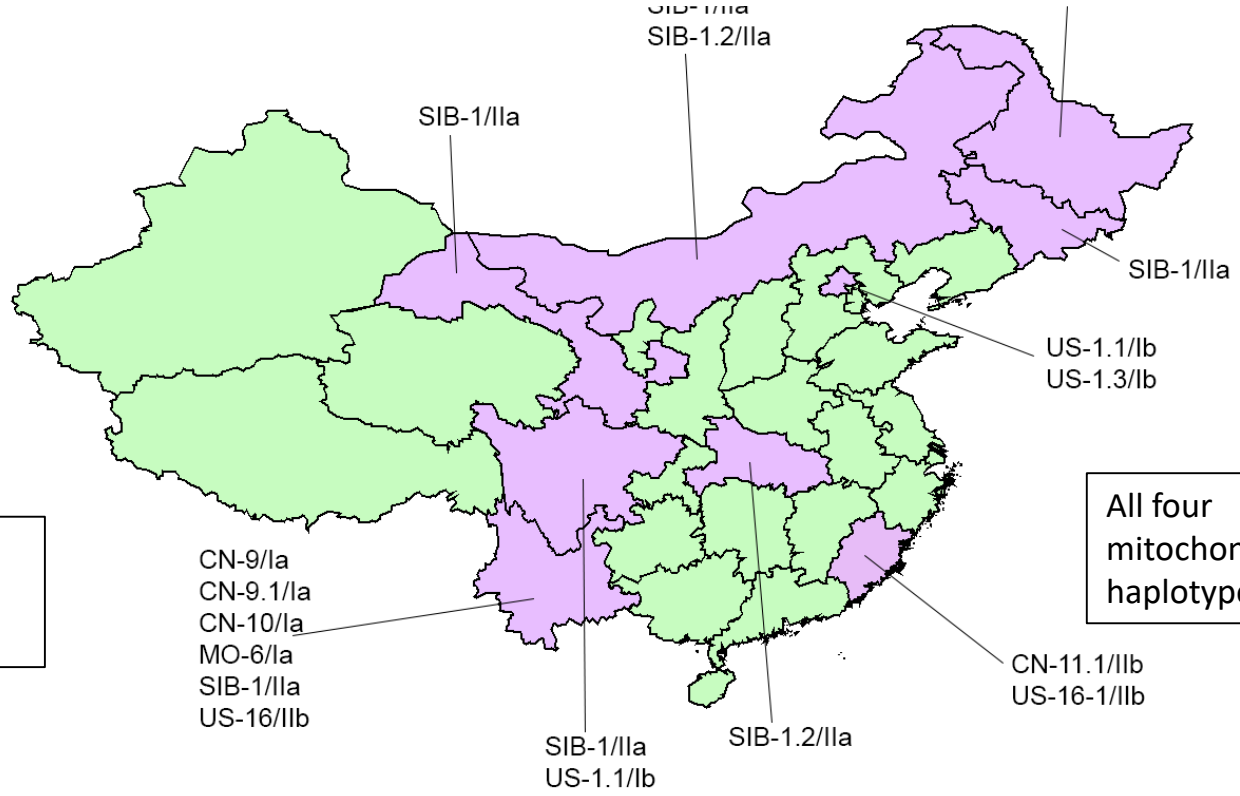


Genetic Structure of *Phytophthora infestans* Populations in China Indicates Multiple Migration Events

Liyun Guo, Xiao-Qiong Zhu, Chia-Hui Hu, and Jean Beagle Ristaino

First and second authors: Department of Plant Pathology, China Agricultural University, Beijing 100193; and third and fourth authors: Department of Plant Pathology, Box 7616, North Carolina State University, Raleigh 27695.
Accepted for publication 12 May 2010.

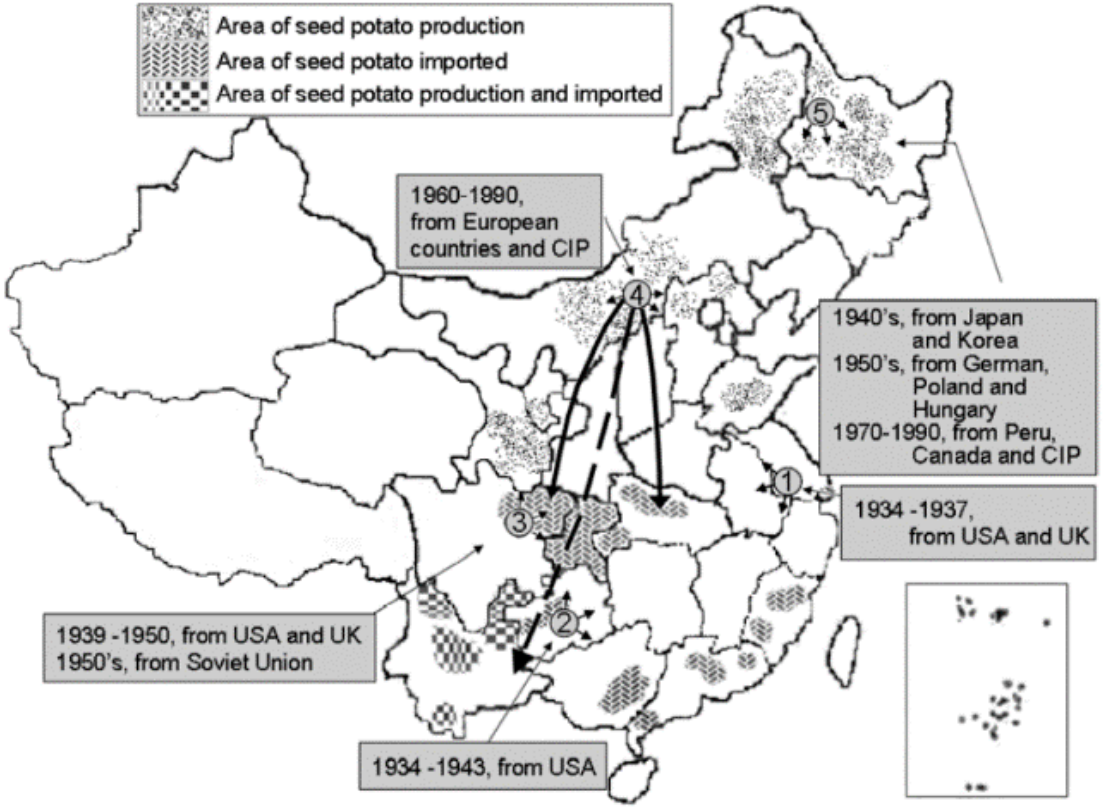
SIB-1 throughout China



More diversity present in southern China

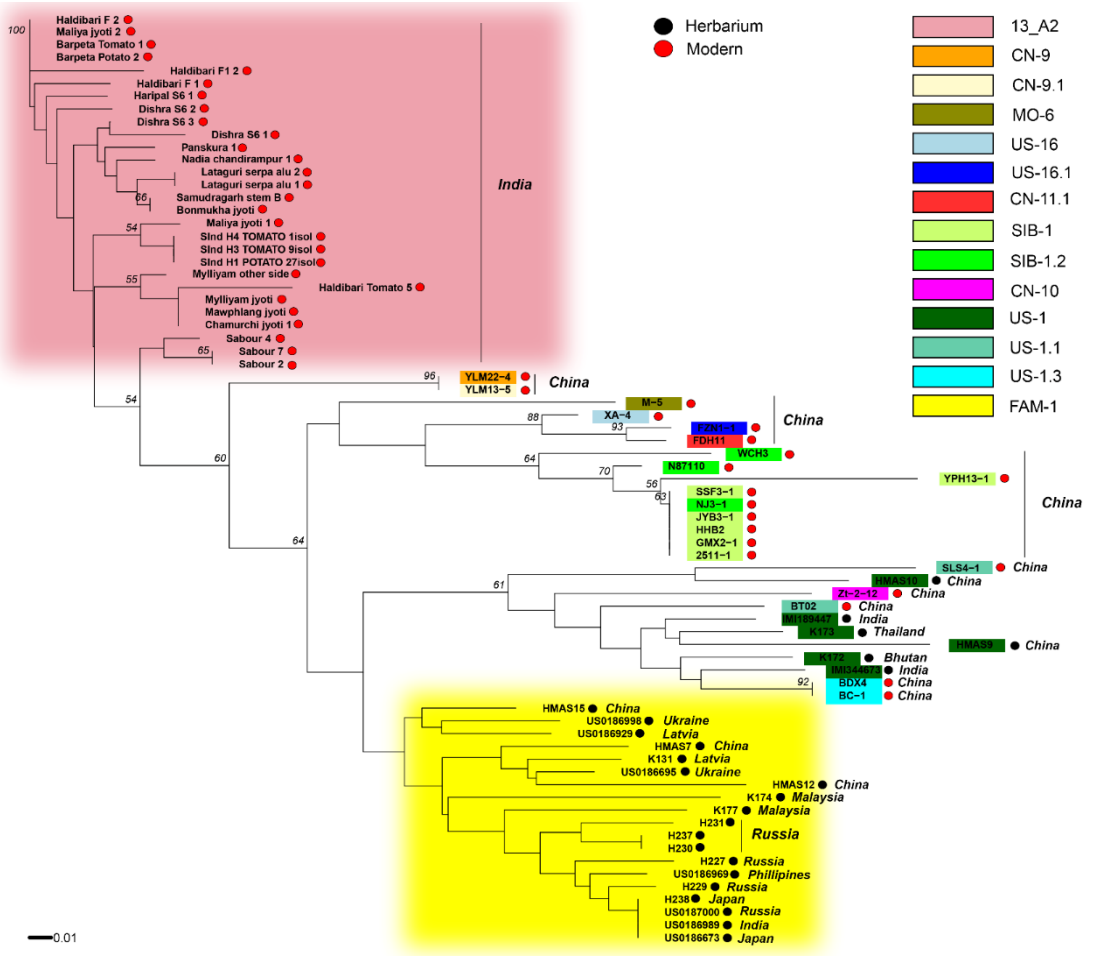
All four mitochondrial haplotypes present

Liyun Guo et al 2010 – Chinese populations



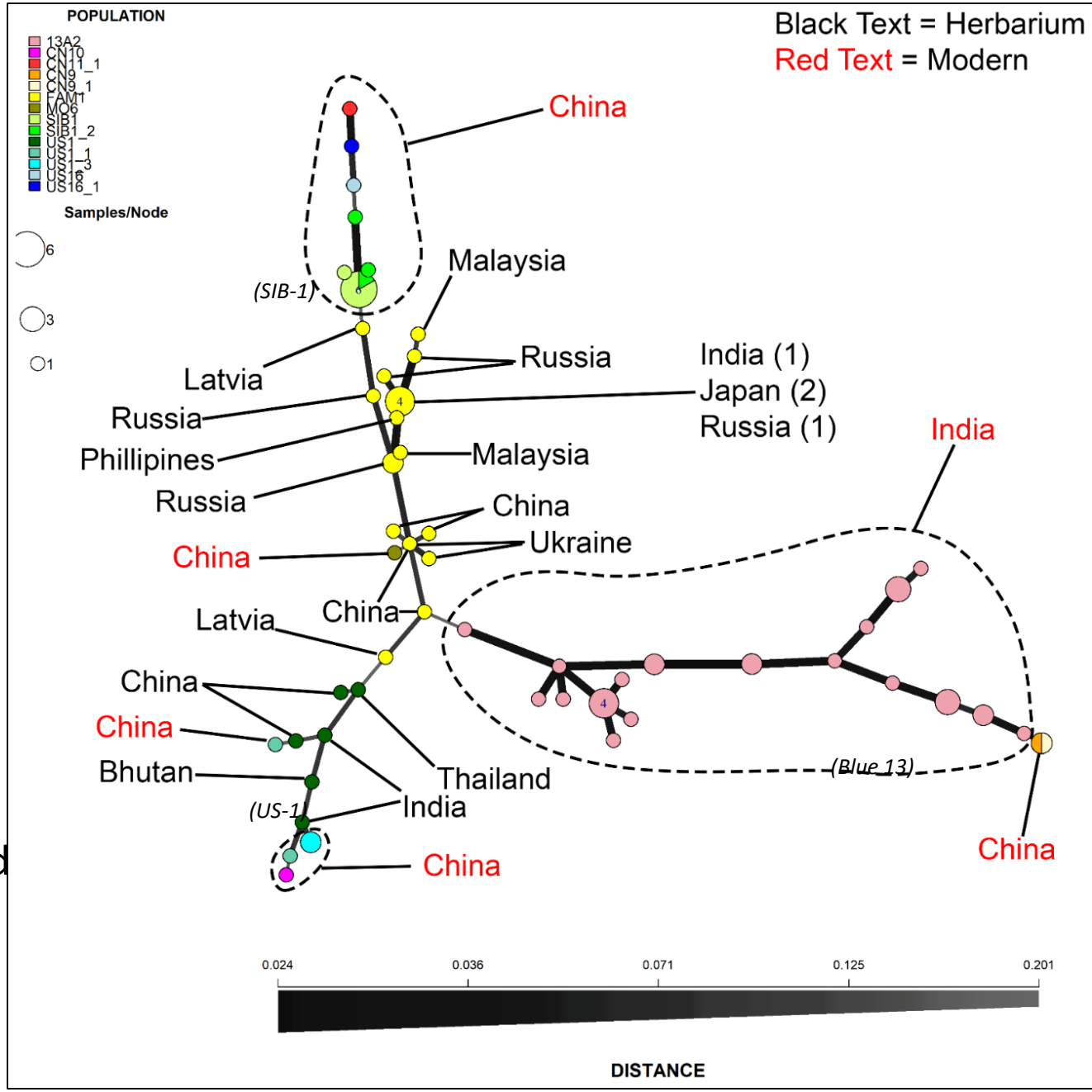
Asian baseline SSR data *P. infestans*

- Neighbor joining tree of 7 SSR loci, 1000 bootstrapping repetitions
- FAM-1 was oldest lineage and widespread in Asia. Shows no strong grouping by geography- was widespread
- US-1 migrated – mid 20th century- China, India, Bhutan, Thailand
- SIB-1 dominant lineages 1998-200 China. First found in Russia
- MO-6 - clusters with CN-11, US-16 -modern Chinese lineages than FAM-1
- More recent aggressive lineages – Blue 13 – China, India and elsewhere



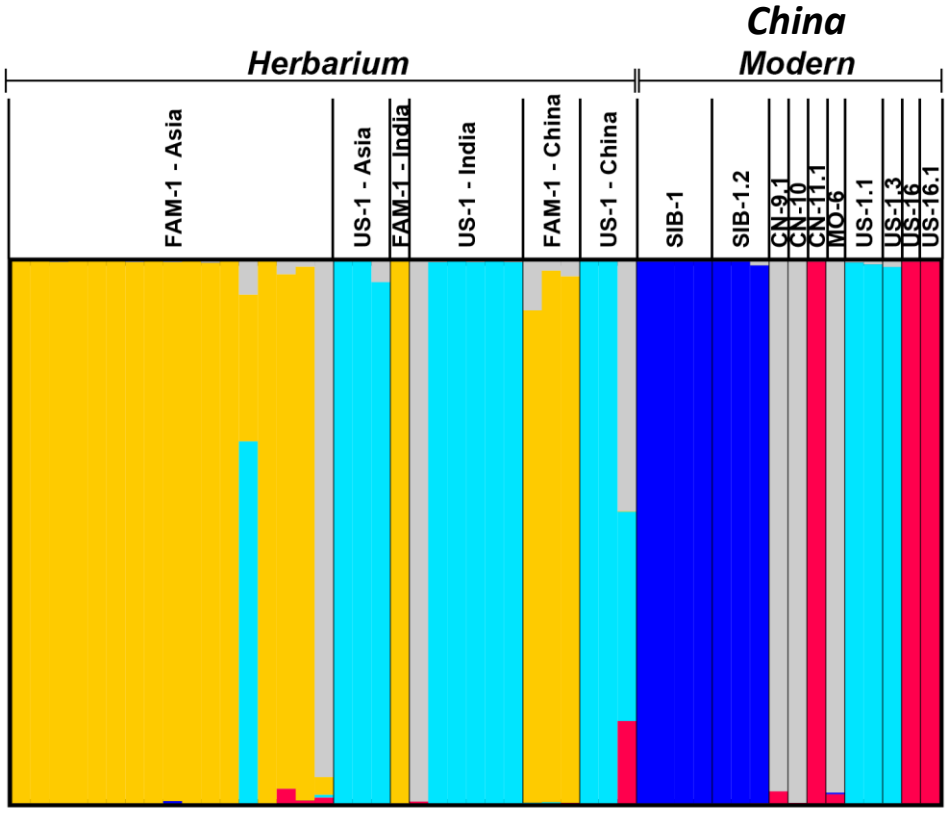
MSN Asian lateblight

- Minimum spanning network of 7 SSR loci
- SIB-1 and variants form group with CN-11.1
- 13A2 forms group with CN-9 and variant
- CN-10 closely associated with US-1 and variants
- MO-6 in close proximity to FAM-1 samples



Asia SSR baseline data

- Structure analysis of 12 SSR loci (clone corrected, no admixture model)
- Optimal K=5 based on Structure Harvester
- SIB-1 and variants formed unique group
- CN-11.1 and US-16/16.1 formed group
- CN-9.1/10, MO-6, formed group that showed some similarity to a couple of herbarium samples
- I will deposit baseline lineage DNA with CIP China



Improve plant disease diagnostic clinics and training globally

- Workshop June 2011 - 25 students – 9 countries, gender and ethnic diversity in selection – Univ. Costa Rica
- Sept 29-Oct 4, 2013 at Pan American University, Zamorano, Honduras
- Sept 2015, Bangalore India
- Oct. 10-15, 2015, National Taiwan Univ. Tapei
- May 8-20, 2016 BecA Hub Kenya
- May 2018, Univ. Catania Sicily

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Future research and capacity building

Population genotyping

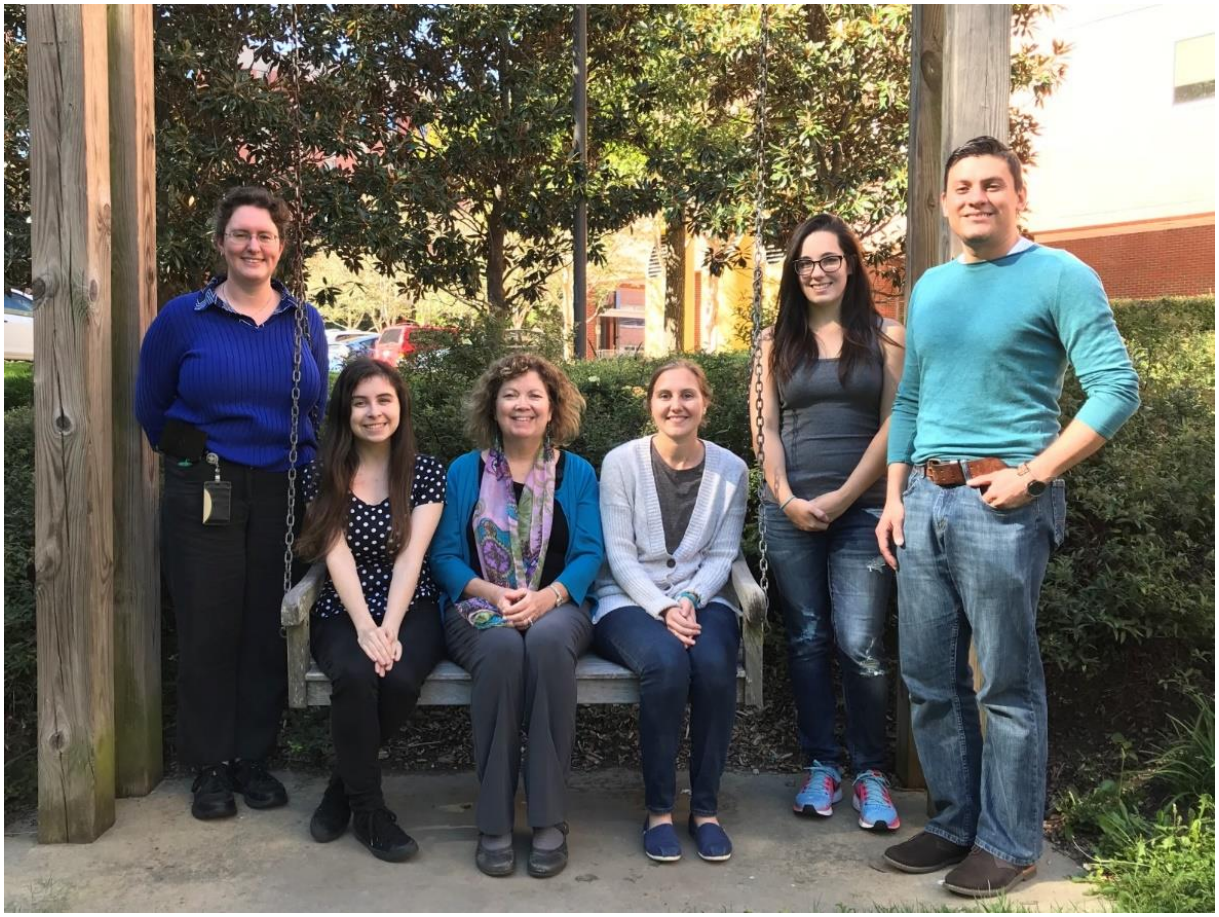
- Expand Phytophthora Diagnostics Training to include bionformatics module. Conduct genotyping and bioinformatics workshop. Use actual data sets – “Phytophthora hack-a-thon”
- Ran a bioinformatics workshop CIP – Peru, 2016, provided SA SSR standards
- Provide DNA standards to other global network of SSR labs (Africa, Asia)
- Start a GlobalBlight Network.
- Continue to train individuals and labs to run SSR genotyping- build human capacity
- Develop an open database that can be queried for identification of major SSR lineages –North America, Mexico, and South America. Africa, Asia

Detection:

- Link sensor detection to real-time monitoring
- Genotype sensor
- Need fast geospatial mapping of late blight

Risk- based modeling using climate data

- Analyze US outbreak data using weather modeling- Chris Jones, Center for Geospatial Analytic
- Link sensors with weather models



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J. Ristaino's laboratory website

http://ristainolab.cals.ncsu.edu/

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